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THE EFFECTS OF SOME AMIDO-ACIDS ON THE DEVELOPMENT OF THE EGGS OF ARBACIA AND OF CHÆTOPTERUS.

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In 1909, Mathews published a short account of some experiments which he made to ascertain the effects of various amido-acids on the development of the eggs of *Arbacia*. The results of these experiments have considerable theoretical interest, since they seem to show that the course of embryonic development can be determined, to a greater or a less extent, by these products of protein digestion.

While I was working in the Marine Biological Laboratory at Woods Hole, Mass., in the summer of 1909, Dr. Mathews kindly furnished me with a number of amido-acids in order that I might repeat and extend his experiments and make a detailed study of the different types of larvæ that might be obtained. As it seemed worth while to determine whether amido-acids can alter the course of development in various kinds of eggs or whether they have a specific action on the eggs of *Arbacia*, the experiments were carried beyond the limits originally intended and were made with the eggs of an annelid, *Chætopterus pergamentaceus*, as well as with the eggs of the sea-urchin, *Arbacia punctulata*.

In addition to cystin, leucin and tyrosin, the three amido-acids which Mathews used in his experiments, both kinds of eggs were subjected to the action of glutamic acid, aspartic acid, asparagine, glycocoll and alanin. In each series of experiments eggs from two or more females were thoroughly mixed and then artificially fertilized in sea-water. As soon as the polar bodies had been

extruded, approximately equal portions of the eggs were transferred into finger bowls which contained 100 c.c. of the solution to be tested. As a control by which to judge of the effects of the solutions, one portion of the eggs was allowed to develop in 100 c.c. of normal sea-water. The various experiments were made in a similar manner and the eggs were kept under like conditions of light and of temperature during their development in order that the results of the experiments might not be affected by environmental conditions other than those that were being studied.

A. EXPERIMENTS WITH THE EGGS OF *Arbacia punctulata.*

As the breeding season of *Arbacia* is near its close the latter part of July, only a small number of eggs suitable for experimental purposes could be obtained. All of the eggs used were presumably in a normal physiological condition, as at least 90 per cent. of those in the control cultures developed in a normal manner and became plutei.

In each series of experiments observations were made at frequent intervals on the living embryos. These observations were later supplemented by a microscopic study of various lots of material that had been fixed in corrosive sublimate and stained with Heidenhain's iron-hæmatoxylin or with Delafield's hæmatoxylin followed by eosin.

Cystin ($C_6H_{12}O_4N_2S_2$).—As this substance is very insoluble in cold sea-water, the solution used in the first experiment that was made was prepared in the following way: A quantity of the pure crystalline salt was placed in a flask of sea-water heated to 40° C. The mixture remained at this temperature for one half hour and was then sealed and set aside. After three days the solution was filtered, to remove the undissolved cystin, and used within a few hours.

A lot of *Arbacia* eggs was fertilized at 11.45 A.M. on the morning of July 14, 1909, and a portion of them was placed in the saturated solution of cystin at 12.15 P.M. These eggs were found to be segmenting in a normal manner when division of the eggs in the control culture took place at 12.50 P.M., and for some hours the eggs of both cultures seemed to be developing at about

the same rate. If the cystin had any effect on the segmentation it was too slight to be detected either in the living eggs or in preserved material.

On the morning of July 15, both cultures contained many living embryos; those of the control were well-developed gastrulæ that were swimming at the surface of the water in a normal manner; those in the cystin solution were decidedly smaller than the control larvæ, and most of them were swimming at the bottom of the dish. Thirty hours after the experiment was started all of the larvæ in the cystin solution were dead, although the larvæ in the control culture were still in good condition. Preserved material showed that the development of the eggs that had been subjected to the action of the cystin solution took place in a perfectly normal manner, although it was somewhat slower than that of the eggs in the control lot.

Mathews found that cystin produced a decided acceleration in the development of the eggs of *Arbacia*, which was apparent from the fourth division on. The solution that he used was made as follows: "One hundred centimeters of sea-water were shaken for a moment with about a centigram of crystalline cystin and the mixture poured into a finger bowl with the undissolved cystin. The eggs, fertilized something less than an hour before, were then added and the eggs lay during development among the crystals of cystin at the bottom of the dish." As a solution made in this way is undoubtedly much weaker than that employed in my first experiment, it seemed probable that the opposing results obtained by Mathews and myself might be due to the difference in the strength of the solutions to which the eggs were subjected. The experiment was therefore repeated with a different lot of eggs, the solution of cystin that was used being prepared in the manner described by Mathews.

In this experiment, also, the development of the eggs appeared to progress at about the same rate in both the cystin culture and in the control. Some of the eggs in the cystin solution seemed to segment much more rapidly than others, and a very few of them developed at a faster rate than the major portion of the eggs in the control culture. A careful comparison between the two cultures, made at intervals of about one half hour during the

entire day, failed to show any marked acceleration in the development of the great majority of the eggs in the cystin solution. Twenty hours after the experiment began swimming larvæ were found at the surface in both cultures, so in this instance the development of the blastulæ was not retarded by the cystin. The solution was ultimately harmful, however, as all of the larvæ in the cystin culture died within thirty-six hours, while those of the control developed into plutei that lived for several days. No unusual types of larvæ were noted among the living forms, and none were found in microscopic preparations of the older embryos.

The *Arbacia* eggs with which Mathews experimented were undoubtedly in a very different physiological condition from those that I used, for Mathews states that in the control lots for his experiments "hardly a pluteus was to be found and these few were generally abnormal." In both of my control cultures the great majority of the eggs formed normal plutei that lived for some days. With such a great difference in the lots of eggs experimented upon it is not surprising that the results do not agree, since the reaction of eggs to any external stimulus depends, to a considerable extent, upon the particular physiological conditions existing in the eggs at the time that the stimulus is applied.

Leucin ($C_6H_{13}NO_2$).—By the use of a weak solution of "impure leucin" Mathews changed the course of development of the eggs of *Arbacia* so that many of the embryos were totally unlike *Arbacia* larvæ. "In many, evagination of the entoderm instead of invagination, took place. A few developed a ciliated band in the shape of the star-fish bipinnaria. . . . Another form was perfectly spherical with a single ciliated band about the middle. It looked in its external form like a small trochophore." Unfortunately, it was not possible to obtain any of the impure leucin with which Mathews produced these remarkable forms of *Arbacia* larvæ, and the leucin with which I experimented was presumably pure.

Solutions of various strengths (2, 1, $\frac{1}{4}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) were used on batches of eggs that were fertilized at 11.30 A.M. on the morning of July 16, 1909. The eggs in all of the cultures began segmenting at the same time as those in the control lot,

but the stronger solutions very soon proved toxic and greatly retarded development. None of the eggs in the 2 per cent. solution of leucin had developed beyond the 2-cell stage at the time that the great majority of the eggs in all of the other solutions, as well as in the control, were in the 8-cell stage. A solution of this strength, however, does not kill the eggs quickly, as twenty hours after the experiment began this culture contained a few ciliated larvæ that were much smaller, and less active, than those of the control lot. Within twenty-four hours all of the larvæ in the 2 per cent. solution of leucin were dead.

A microscopic examination was made of a large number of eggs taken from the 2 per cent. solution of leucin at different stages in their development. Many of the young eggs were abnormal in that there was an irregular distribution of the chromosomes to the poles of the segmentation-spindle or a very unequal division of the blastomeres. Such abnormal eggs evidently died before reaching the blastula stage, as nearly all of the older embryos that were examined were normal although somewhat smaller than those of the control culture. A few abnormal blastulæ were found among the older larvæ, but as these larvæ showed only such irregularities of form as may be found in individuals of almost every control culture of *Arbacia* larvæ developing in a small amount of sea-water under laboratory conditions, they could not be considered as due to the specific action of the leucin in changing the course of development.

The eggs in the 1 per cent. solution of leucin began to show the injurious effects of the solution after the first hour, and from this time on their development, although normal, lagged behind that of the control: the weaker solutions had apparently no effects on the early segmentation. The blastulæ in the control culture began moving about fifteen minutes sooner than the larvæ in the other cultures, so evidently all of the leucin solutions retarded development somewhat after the first two or three hours. Plutei that seemed perfectly normal, and that lived for several days, developed in all of the weaker solutions. An examination of a considerable number of these embryos, preserved at various stages in their development, failed to show any larvæ that were in any way comparable to the unusual types that Mathews obtained with impure leucin.

A second experiment was made with leucin on July 24, 1909. In this instance a solution of the strength of ½ per cent. was employed, since stronger and weaker solutions do not alter the course of development. From the beginning of the experiment the segmentation of these eggs lagged behind that of the eggs in the control lot, and the retardation in development was fully as great as that produced by the 1 per cent. solution of leucin in the former series of experiments. Later the development of these eggs progressed at a more normal rate, and after seven hours the embryos appeared nearly as well developed, and fully as vigorous, as those in the control. The next morning larvæ were swimming at the surface in both cultures, but those in the leucin solution soon dropped to the bottom of the dish and began to disintegrate. Microscopic preparations showed that the very great majority of these larvæ were normal in every respect.

Mathews states that in the summer of 1908, when his experiments were made, the sea-urchin eggs showed in many instances the remarkable peculiarity, recorded by Mathews and Whitcher ('03), that "a large number of eggs while living for several days not forming plutei, or but a small per cent. of irregular plutei." The experiments which Mathews made to test the action of amido-acids on the development of the eggs of *Arbacia* were made therefore, wholly or in great part, on eggs that were in a peculiar physiological condition when experimented upon: whether they could be considered as normal is doubtful. The unusual types of larvæ that Mathews obtained by treating eggs with a weak solution of impure leucin were probably due to abnormal or unusual conditions existing in the eggs at the time of their fertilization, and not to the specific actions of leucin in changing the course of development. The effects of leucin on eggs of *Arbacia* that are in a normal physiological condition when fertilized depends chiefly upon the strength of the solution used: a strong solution retards development and causes the early death of the embryos; a weak solution permits of normal development at first and is toxic only after many hours.

Tyrosin ($C_9H_{11}NO_3$).—This substance is not very soluble in cold sea-water, and in order to obtain a solution of sufficient strength one gram of tyrosin crystals was put into 100 c.c. of sea-water and

the mixture brought to the boiling point. The solution was then cooled to laboratory temperature, filtered, and used at once.

The early development of the eggs used in this experiment was normal, although slightly delayed. After twenty hours ciliated larvæ were present in great number in the solution, but they were moving feebly and beginning to show degenerative changes. Prepared material showed that tyrosin had retarded the development of the eggs but produced no abnormalities. These results agree with those obtained by Mathews in a similar experiment.

Glutamic Acid ($C_5H_9NO_4$).—Various solutions of this substance (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) were used on the eggs of *Arbacia*, and all of them proved to be injurious from the beginning of the experiment. The eggs placed in the stronger solutions (1 and ½ per cent.) were killed at once. A few of the eggs subjected to the action of the $\frac{1}{10}$ per cent. solution began to segment in a normal manner, but none of them developed beyond the early stages of segmentation. The eggs in the $\frac{1}{30}$ per cent. solution continued to live for some time, but their development was very greatly retarded and stopped entirely when the gastrula stage was reached. Preparations of these eggs showed that the effects of the glutamic acid was to check development, not to produce unusual types of larvæ.

Aspartic Acid ($C_4H_7NO_4$).—This substance has a more deleterious action on the eggs of arbacia than has glutamic acid. All of the eggs placed in a 1 per cent. solution and in a ½ per cent. solution were killed at once; those subjected to the action of a $\frac{1}{10}$ per cent. solution did not develop beyond the 2-cell stage. A solution of the strength of $\frac{1}{30}$ per cent. allowed a considerable number of the eggs to develop to the blastula stage, but segmentation was very irregular and much slower than that of the eggs in the control culture.

Preparations of various lots of eggs that had been treated with aspartic acid solutions showed abnormal conditions not found in any of the *Arbacia* eggs subjected to the action of other amido-acids. Most of the eggs that had been subjected to the action of a $\frac{1}{10}$ per cent. solution of aspartic acid for four hours before fixation were found to be still unsegmented, and many of them had been entered by several spermatozoa. Only one sperm-

nucleus had fused with the egg-nucleus, however, and the segmentation-spindle that was formed usually appeared normal, although in many cases it occupied a very eccentric position close to the periphery of the egg. All of the accessory spermatozoa at this time were in the form of a small, rounded nuclei that were scattered throughout the cytoplasm.

The $\frac{1}{30}$ per cent. solution of aspartic acid had a different action on different eggs, depending, doubtless, upon the condition of the eggs when they were placed in the solution. Five hours after the experiment was begun about one fourth of the eggs were still unsegmented; some of the eggs were just beginning to segment; while others were in later stages of segmentation, and the cleavage planes were coming in very irregularly in many cases. A very few eggs had reached the blastula stage at this time, but they were not as well developed as the eggs in the control lot. After twenty-two hours the number of eggs that had reached the blastula stage was found to be consideraly increased. Development had been checked by this time, however, and the greater number of larvæ appeared as more or less irregular masses of cells that were beginning to disintegrate.

Preparations of this material showed many cases of polyspermy. Some of the unsegmented eggs contained a large multipolar segmentation-spindle formed, evidently, by the fusion of several sperm-nuclei with the egg-nucleus: other eggs contained a segmentation-spindle of the normal size with the chromosomes very unequally distributed to the spindle poles. The condition of these eggs greatly resembled that which O. and R. Hertwig ('87) found could be induced in fertilized echinoderm eggs by subjecting them to the action of various chemical substances which prevented their normal development.

Asparagine ($C_4H_{10}N_2O_4$).—This amide of aspartic acid proved to be far less injurious to the eggs of *Arbacia* than did the latter substance, when used in solutions of the same strength (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.). The great majority of the eggs in all of the cultures began to segment at the normal time and in a normal manner. After two hours the eggs in the 1 per cent. solution showed evidence of retarded development, but the eggs in all of the other solutions developed at a normal rate for some hours.

Twenty-four hours after the experiment began, ciliated larvæ were present in great numbers in all of the solutions, but they all died many hours before the death of the larvæ in the control culture.

Glycocoll ($C_2H_5NO_2$).—This substance, which is the simplest of the amido-acids, was much less harmful to the eggs of *Arbacia* than were any of the other amido-acids used in these experiments. During the first twenty-four hours the development of the eggs did not appear to be affected in any way by the solutions used (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.), but during the second day the embryos began to show degenerative changes, and all of them died about fifty hours after the experiment began. Sections of these eggs fixed at various stages of development merely confirmed the observations on the living forms, as no unusual types of larvæ were found.

Alanin ($C_3H_7NO_4$).—This amido-acid dissolves readily in cold sea-water, and it was used in solutions of the following strengths: 2, 1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent. The stronger solutions (2, 1 and $\frac{1}{2}$ per cent.) retarded development from the beginning: the weaker solutions had no apparent effects on the segmentation of the eggs. After twenty-four hours each of the solutions contained a large number of swimming larvæ, and only those in the 2 per cent. solution showed any evidence of retarded development. The embryos in all of the cultures died some hours before the death of the control larvæ, so weak solutions of alanin cannot be considered as favorable media in which to rear the eggs of *Arbacia*. Preserved material showed no abnormalities worthy of note at any stage of development.

All of the amido-acids used in this series of experiments with the eggs of *Arbacia* proved to be toxic, the injurious effects of any substance depending very largely upon the strength of the solution used. In no case was the course of development altered in a definite direction, except in the very young eggs and in these the abnormalities produced were of the types commonly found when fertilized eggs of the sea-urchin are treated with various chemical solutions.

B. EXPERIMENTS WITH THE EGGS OF *Chætopterus pergamentaceus.*

As the eggs of *Chætopterus* could be obtained in considerable numbers at Woods Hole in the summer of 1909, experiments were made to study the influence of amido-acids on the early development of this annelid, in the hope that some definite alterations in development might be produced comparable to those obtained by Loeb ('01) and by Lillie ('02) when eggs of *Chætopterus* were treated with potassium salts. Material intended for microscopic study was preserved in Boveri's picric-acetic solution and stained with hæmatoxylin.

Cystin.—On the morning of August 6, 1909, a lot of *Chætopterus* eggs was placed in 100 c.c. of a saturated solution of cystin as soon as the polar bodies had been extruded. The early development of these eggs was slightly accelerated, and swimming larvæ were found in this culture nearly one half hour before any movement could be detected in the control larvæ. The next day the cystin solution was swarming with well-developed trochophores, but they all died about fifty hours after the experiment began. No abnormal embryos were noted at any stages of development and none were found in preserved material.

The experiment was repeated several days later with eggs from another female. The results obtained were practically the same as in the first experiment, since there was more rapid development during the segmentation period. The solution proved to be toxic after thirty hours, however, killing the embryos without producing any alterations in structure.

Leucin.—In one series of experiments this substance was used on the eggs of *Chætopterus* in solutions of the following strengths: $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{80}$ per cent. None of these solutions had any marked effects on the early segmentation of the eggs, but they evidently caused a slight acceleration in development during a later period as the larvæ in all of the solutions began moving some thirty minutes before there was any movement of the control larvæ. Twenty hours after the experiments were started all of the cultures were carefully examined. The majority of the eggs that had been treated with the $\frac{1}{2}$ per cent. solution had stopped their development in the blastula stage, and were lying at the bottom of the dish apparently dead; a very few larvæ were swimming

at the surface of the solution, but they had evidently reached their maximum development and would soon die. The $\frac{1}{10}$ per cent. solution contained a considerable number of swimming larvæ, but these larvæ were not in good condition and plainly showed the injurious effects of the leucin. A large number of ciliated embryos were found in the $\frac{1}{30}$ per cent. solution, and they appeared somewhat further advanced in development than those in the control culture. Degenerative changes appeared in these larvæ in about twenty-four hours, however, and all of them were dead within thirty hours. No unusual types of larvæ were found in preparations of these eggs fixed at various stages in their development.

As it seemed possible that the solutions of leucin employed in the experiments described above might have been too weak to produce any alteration in the development of the eggs, a second experiment was made in which a batch of eggs was subjected to the action of a 1 per cent. solution of leucin. These eggs segmented at the normal time, but two hours later their development was found to be lagging behind that of the eggs in the control culture. After four hours the retardation in development was very marked, and in some instances two or more eggs had fused together. Loeb and Lillie have noted that the fusion of several embryos into giant forms is a phenomenon of frequent occurrence when eggs of *Chætopterus* are treated with potassium salts. In twenty hours all of the larvæ were dead, and so disintegrated that it was impossible to preserve any material fit for study. Sections of eggs fixed in earlier stages of development failed to show any abnormalities except the occasional fusion of two or more embryos.

Tyrosin.—This substance was used on the eggs of *Chætopterus* in a saturated solution which is less than $\frac{1}{10}$ per cent. Only a very few of the eggs had segmented when the first division occurred in the control eggs. After four hours the tyrosin culture showed all stages in development from the unsegmented egg through to late segmentation, the most advanced eggs being apparently at the same stage of development as the eggs of the control. All of the embryos in the tyrosin solution died within twenty-four hours after the experiment was started. Preserved

material showed that tyrosin acts on the eggs of *Chætopterus* as it does on the eggs of *Arbacia*, causing a marked retardation in development but producing no specific abnormalities.

Glutamic Acid.—Solutions of various strengths (1, $\frac{1}{2}$ and $\frac{1}{10}$ per cent.) were used, the eggs being placed in the solutions about three quarters of an hour after their fertilization. All of the eggs in the two stronger solutions were evidently killed at once as none of them made any attempts to divide. Some of the eggs in the $\frac{1}{10}$ per cent. solution began to elongate after the solution had acted upon them for one hour, and later many of these eggs took on an irregular shape as if attempting to divide into several cells at the same time. None of these eggs had segmented after five hours, however, so they were all returned to normal sea-water in the hope that they would then be able to continue their development. There was no segmentation of any of the eggs, although they appeared to live for some hours.

Sections of preserved material showed that the segmentation-spindle had formed in many eggs in an apparently normal manner, but that development had been stopped at this point.

Aspartic Acid.—Eggs of *Chætopterus* fertilized at 10.55 A.M. on August 8, 1909, were placed in solutions of aspartic acid (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) at 11.25 A.M. The eggs in the control culture were segmenting at 11.55 A.M., but no evidence of cleavage could be detected in any of the eggs in the aspartic acid solutions until 1.30 P.M., when a few of the eggs in the $\frac{1}{30}$ per cent. solution began to elongate as if about to divide. A number of these elongated eggs were isolated and carefully watched for some time, but in no case did any division occur. Sections of preserved material showed that some eggs contained a normal segmentation-spindle, while others had a multipolar spindle that occupied an eccentric position close to the periphery. The stronger solutions of aspartic acid killed the eggs before the formation of the segmentation-spindle.

Asparagine.—Solutions of this substance of the same strengths as those used in the experiments with aspartic acid were tested. Normal cleavage began in the eggs of all of the cultures at the same time as in those of the control lot. Observations made at frequent intervals during the next four hours showed that seg-

mentation was progressing in a normal manner and at about the same rate in all of the solutions.

Five hours after the eggs had been fertilized a few larvæ in the $\frac{1}{10}$ per cent. solution were moving slowly: at this time there was no movement of any of the embryos in the other cultures or in the control lot. A weak solution of asparagine, therefore, slightly accelerates the development of the eggs of *Chætopterus*, if it be that an earlier movement of the embryos is indicative of a more advanced stage of development. At the end of the sixth hour the effects of the various solutions were very marked: the embryos in the $\frac{1}{10}$ per cent. solution were moving more actively than those in the control, and they seemed slightly better developed; the larvæ in the other solutions were moving slowly and their development lagged considerably behind that of the control larvæ. After eight hours the larvæ in the 1 per cent. solution were all at the bottom of the dish and evidently dying; no abnormal types of larvæ could be detected among the living forms, and none were found in preserved material that was examined later. The embryos in the other solutions were swimming at the surface after ten hours, but none of them lived more than twenty-four hours.

Glycocoll.—In the strengths of solutions used (1, $\frac{1}{2}$ and $\frac{1}{10}$ per cent.), this substance did not appear to have any effects whatever on the eggs during the first twelve hours. On the second day the larvæ began dying, and all of them had been killed by the end of the third day.

Alanin.—Batches of *Chætopterus* eggs that had been artificially fertilized at 10.30 A.M. on the morning of August 8, 1909, were put into various solutions of alanin (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) at 11 o'clock. The eggs in all of the cultures, including the control, began segmenting at the same time, and all of them developed at about the same rate during the next two hours. At 3.30 P.M. a number of swimming larvæ were found in the $\frac{1}{10}$ and in the $\frac{1}{30}$ per cent. solutions, but at this time there was no movement of the larvæ in any of the other cultures. At 4.30 P.M. ciliated larvæ were present in great numbers in all of the solutions; but the larvæ in the 1 per cent. solution could move but slowly, and soon all of them sank to the bottom of the dish and disintegrated.

At 9 A.M. on the morning of August 9, the larvæ in the ½ per cent. solution were dying, and a number of giant embryos had been formed by the fusion of two or more of the larvæ: the embryos in the $\frac{1}{10}$ per cent. and in the $\frac{1}{30}$ per cent. solutions were apparently normal and were moving vigorously. All of the larvæ were dead on the morning of August 10, although the trochophores in the control culture were still very active at this time. Preserved material showed no abnormalities worthy of note.

As weak solutions of alanin did not seem to affect the early development of the eggs adversely a second series of experiments was made in which batches of *Chætopterus* eggs were treated with 4 per cent. and with 2 per cent. solutions of alanin as soon a they had extruded their polar bodies.

None of the eggs in the 4 per cent. solution segmented, and sections of preserved material showed that the eggs had been killed before the formation of the segmentation-spindle. When cleavage began in the eggs of the control lot at 11 A.M. a very few of the eggs in the 2 per cent. solution were dividing in an apparently normal manner; in the great majority of the eggs segmentation was very greatly delayed. After four hours only a few eggs had reached the 4-cell stage, and in these eggs the cleavage planes had come in very irregularly. An hour later development had stopped entirely and the eggs were fusing into large, irregularly shaped masses. At this time the eggs were transferred into normal sea-water in the hope that segmentation might be resumed, but although the eggs seemed to live for some hours, none of them developed beyond the 4-cell stage.

In microscopic preparations of eggs that had been in the 2 per cent. solution of alanin for two hours before fixation only a very few normal 2-cell stages were found, and the great majority of the eggs contained a multipolar spindle with the chromosomes very irregularly distributed along the spindle fibres. Material fixed after the solution had acted for five hours showed that only the first cleavage in any of the eggs was normal and that in most eggs development had stopped at this point. Where further division had occurred the blastomeres were very irregular in size and shape, and although hundreds of eggs were examined no stage later than an 8-cell stage could be found.

When multipolar spindles formed in the eggs as a result of their treatment with a 2 per cent. solution of alanin the eggs, apparently, were never able to divide, although there seemed to be a long period during which active and resting stages alternated with each other. In the resting stages the eggs contained either one large, oblong nucleus, or several smaller ones that were more or less irregular in outline. In the active periods one large, multipolar spindle with hundreds of chromosomes scattered about it would be formed, or several small spindles, all more or less irregular, would be scattered throughout the cell. In some of these eggs a number of accessory asters were formed, similar to those that Morgan ('96, '99) found could be produced in the eggs of *Arbacia* and of various other forms by means of salt solutions.

A 2 per cent. solution of alanin produced greater abnormalities in the eggs of *Chætopterus* than did any of the other solutions of amido-acids that were used, but as these abnormalities were of the types that can be produced in different kinds of eggs by treatment with various salts they cannot be considered as the result of any specific action on the part of the alanin.

Summary and Conclusions.

With the exception of cystin, which is a sulphur-containing compound, all of the amido-acids used in these experiments are composed of the same chemical elements, yet they differ to a marked extent in their toxic action on developing eggs. Glutamic acid and aspartic acid are by far the most injurious, even a $\frac{1}{30}$ per cent. solution of these substances killing the eggs of both *Arbacia* and of *Chætopterus* at a very early period. Glycocoll, on the other hand, permits of the development of normal plutei and trochophores, and only injures the embryos after twenty-four hours. The other amido-acids used retard development, to a greater or less extent, depending chiefly upon the strength of the solution employed.

A brief summary of the effects of the various solutions of amido-acids on the development of the eggs of *Arbacia* and of *Chætopterus* during the first twelve hours is given in the following table. Ultimately all of the solutions are toxic, even though they appear to favor development during an early period.

TABLE I.

Amido-acid.	Solution Used.	Effects on *Arbacia* Eggs.	Effects on *Chætopterus* Eggs.
Cystin	Saturated	No effects on segmentation; later development retarded.	Development accelerated.
	$\frac{1}{30}$ per cent.	Development very slightly retarded.	Development slightly accelerated.
	$\frac{1}{10}$ per cent.	Development very slightly retarded.	Development slightly accelerated.
	$\frac{1}{4}$ per cent	.Development very slightly retarded.	.....
Leucin.	$\frac{1}{2}$ per cent.	Development slightly retarded.	Development accelerated at first, but stopped in blastula stage.
	1 per cent.	Development retarded after 1 hour.	Development retarded after 2 hours; embryos fused.
	2 per cent.	Development greatly retarded; a few eggs abnormal.	.....
Tyrosin.	Saturated.	Development retarded.	Development retarded.
	$\frac{1}{30}$ per cent.	Development stopped in the gastrula stage.	.....
Glutamic acid.	$\frac{1}{10}$ per cent.	Eggs killed in early segmentation.	Eggs lived for some time, but no segmentation.
	$\frac{1}{2}$ per cent.	Eggs killed at once.	Eggs killed at once.
	1 per cent.	Eggs killed at once.	Eggs killed at once.
	$\frac{1}{30}$ per cent.	Development stopped in blastula stage; many eggs abnormal.	Eggs lived for some time, but no segmentation.
Aspartic acid.	$\frac{1}{40}$ per cent.	Development stopped at 2-cell stage; many eggs abnormal.	Eggs killed at once.
	$\frac{1}{2}$ per cent.	Eggs killed at once.	Eggs killed at once.
	1 per cent.	Eggs killed at once.	Eggs killed at once.
	$\frac{1}{30}$ per cent.	No effects noted.	.....
	$\frac{1}{10}$ per cent.	No effects notes.	Development slightly accelerated.
Asparagine.	$\frac{1}{2}$ per cent.	No effects noted.	Segmentation not affected; later development retarded.
	1 per cent.	Development retarded after 2 hours.	Segmentation not affected; later development retarded.
	$\frac{1}{30}$ per cent.	No effects noted.	No effects noted.
Glycocoll.	$\frac{1}{10}$ per cent.	No effects noted.	No effects noted.
	$\frac{1}{2}$ per cent.	No effects noted.	No effects noted.
	1 per cent.	No effects noted.	No effects noted.
	$\frac{1}{30}$ per cent.	No effects noted.	Development slightly accelerated.
Alanin.	$\frac{1}{10}$ per cent.	No effects noted.	Development slightly accelerated.
	$\frac{1}{2}$ per cent.	Development somewhat retarded.	Segmentation not affected; older embryos fused.

Amido-acid.	Solution Used.	Effects on *Arbacia* Eggs.	Effects on *Chætopterus* Eggs.
Alanin.	1 per cent.	Development greatly retarded.	Development retarded after 2 hours.
	2 per cent.	Development greatly retarded.	Development retarded; many eggs abnormal.
	4 per cent.	.....	Eggs killed at once.

As shown in the above table, all of the stronger solutions of amido-acids that were used had much the same effect on both kinds of eggs experimented upon, but several of the weaker solutions had a much more pronounced action on the eggs of *Chætopterus* than on those of *Arbacia*. Weak solutions of cystin, of leucin, of asparagine and of alanin accelerate the development of the eggs of *Chætopterus* to a noticeable extent, yet none of these solutions have apparently any effect on the early development of the eggs of *Arbacia*. The eggs of *Chætopterus* cannot segment at all when placed in a $\frac{1}{30}$ per cent. solution of aspartic acid, although this solution permits the eggs of *Arbacia* to develop to the blastula stage.

The abnormalities produced in the eggs of *Arbacia* and of *Chætopterus* by various solutions of amido-acids consist chiefly of polyspermy, irregularities in the mitotic figures, variable cleavage, and a fusion of several embryos into giant forms. No embryos were found that showed either the larval characteristics of other forms or marked peculiarities of structure that might be attributed to the specific action of the solution in which they were reared.

The results obtained in these experiments indicate that solutions of amido-acids can alter the rate at which the eggs of *Arbacia* and of *Chætopterus* develop, but that they have no influence whatever in determining the character of the development, when the eggs experimented upon are in a normal physiological condition.

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